Structural Studies on the Mycolic Acids

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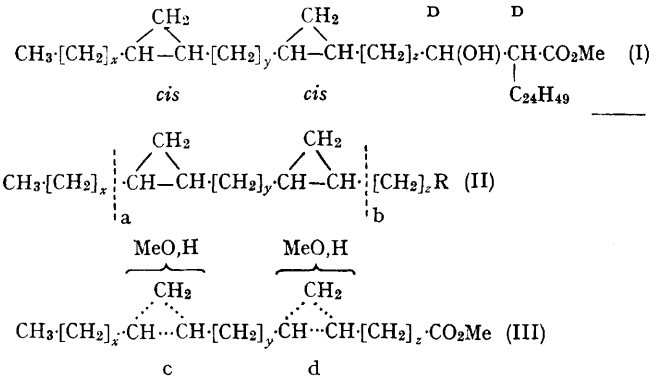
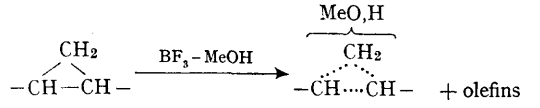
IN earlier Communications[1,2] the general structure (I) was advanced for methyl mycolate-I isolated from human tubercle bacilli (strains D.T., P.N., and C.). This structure, without the stereochemical details, corresponded to the general structure proposed earlier by Etémadi and Lederer[3] for methyl α-mycolate (Test). For the main component of the latter, the structure (I, $x = 17$, $y = 11$, $z = 16$; without stereochemical designations) was derived from a high-resolution study of certain peaks in its mass spectrum; pyrolysis occurred in the mass spectrometer giving rearrangement peaks at *m/e 410*, 382 (straight-chain esters) and 740, *768*, 796, 824 (meroaldehydes) (the most abundant peak in any series is in italics). A series of peaks at *m/e* 459, *487*, 515, 543 were said to be doublets consisting of oxygen-containing and hydrocarbon components arising by cleavage of the meroaldehydes (II; R=CHO) at positions a and b.

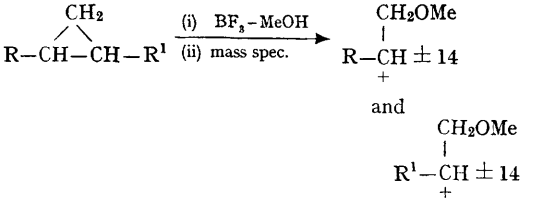
In order to discover whether the same procedure could be applied to methyl mycolate-I, the series of fragments at *m/e* 431, *459*, 487, 515, 543[1] were studied by high-resolution mass spectrometry (by R. T. Aplin; A.E.I., M.S.9).[4] The oxygen-containing fragments were prominent but the hydrocarbon components not significantly larger than the general background of the mass spectrum. Pyrolysis of methyl mycolate-I gave meromycolal-I (II; R=CHO) whose mass spectrum showed molecular ions at *m/e* 712, *740*, 768, 796, 824 and a series of fragments at *m/e* 431, *459*, 487, 515, 543 $(M-281)^1$ corresponding exactly to those observed for the parent ester. Oxidation of this aldehyde with silver oxide[5] followed by esterification gave methyl meromycolate-I (II; R=CO_2Me), whose mass spectrum showed peaks due to the molecular ions at *m/e* 742, *770*, 798, 826, 854, but also indicated the presence of homologues of *m/e* 700, 714, 728, *756*, 784, 812, 840, perhaps formed from oxidative degradation by nitric acid used in working-up the above oxidation product. There were no intense peaks in the mass spectrum of methyl meromycolate-I attributable to simple cleavage adjacent to cyclopropane groups; this is in agreement with the known behaviour of long-chain esters containing isolated cyclopropane groups.[6,7]



Recent work in this laboratory[8] has shown that synthetic long-chain 1,2-disubstituted cyclopropane esters were readily attacked by boron trifluoride–methanol to give a mixture of primary and secondary methoxy-esters and the corresponding olefins as represented by the scheme



The mass spectra of these methoxy-esters contained intense peaks due to ions of the type $R{\cdot}\overset{+}{C}H{\cdot}OMe$ and $R{\cdot}\overset{+}{C}H{\cdot}CH_2{\cdot}OMe$, and it was found that the position of a cyclopropane ring was revealed by the presence of two sets of three homologous ions of similar intensity, *i.e.*,



Treatment of methyl meromycolate-I (II; R=CO_2Me) with boron trifluoride–methanol in dichloromethane gave a dimethoxy-, an unsaturated methoxy-, and an unconjugated diene-ester. The mass spectrum of the latter was similar to that of the parent meromycolate, but the spectra of both methoxy-esters contained peaks showing the location of the original cyclopropane groups. In the case of the dimethoxy-ester (III) cleavage at centre c of the terminal portion gave ions of *m/e* 325, 339, 353 which lead to the value of $x = 19$ for the main component. Cleavage at centre d and elimination of methanol from centre c gave ions of *m/e* 561, 575, 589 from which it may be calculated that $x + y = 34$ and hence $y = 15$ for the main component. The corresponding meroaldehyde was that having *m/e* 740 (*i.e.*, $x + y + z = 44$) which gives $z = 10$. The only other meroaldehyde of comparable abundance was that of *m/e* 768 (70% of *m/e* 740); since the mass spectrum of the dimethoxy-ester (III) contained no significant sets of peaks corresponding to an increase of two units in either x or $x + y$, it follows that for this aldehyde $z = 12$. Ions confirming these values, containing the methoxycarbonyl group, formed by cleavage at centre d and at centre c with elimination of methanol from centre d were observed; however, since meromycolate-I contained homologous artefacts these patterns were not so distinctive. In conjunction with evidence already advanced[1,2] the structures (I, $x = 19$, $y = 15$, $z = 10$) and (I, $x = 19$, $y = 15$, $z = 12$) may be proposed for the main and next most abundant components of methyl mycolate-I, respectively.

In the light of these structures the ready loss of a hydrocarbon fragment of 281 mass units in the mass spectrum of the meroaldehyde-I is remarkable. Such a process does not occur in meromycolate-I or anhydromycolate-I[1] and thus appears to be a property of such long-chain cyclopropane aldehydes, and perhaps involves some intramolecular macrocyclic interaction of the aldehyde group with the remote cyclopropane group.

The procedure used by Etémadi and Lederer[3] to assign their structure for methyl α-mycolate (Test) appears questionable in three respects. Firstly, the hydrocarbon components of the

doublets at *m/e* 459 to 543 would only have significance if their intensities were markedly greater than that of the general hydrocarbon background and paralleled those of the oxygen-containing fragments. In the case of methyl mycolate-I this was not so[4] and the French workers did not record these intensities for methyl α-mycolate (Test). Secondly, in order to make any use of their method, previously applied to methyl α-kansamycolate,[9] it was necessary to correlate the fragment of *m/e* 487 with the aldehyde of *m/e* 740, and not that of *m/e* 768 which had in fact the most intense peak.[3] The main component of α-meromycolate (Test) (*m/e* 770) corresponded to the required aldehyde (*m/e* 740) but this ester was obtained after an oxidation similar to that which in the preparation of methyl meromycolate-I resulted in some degradation from the carboxyl end. A preparation of α-meromycolal (Test) was described[3] but its mass spectrum which would have clarified this point was not reported. Thirdly, if simple cleavage adjacent to 1,2-disubstituted cyclopropane groups was the correct cause of the fragmentation of the meroaldehyde in the mass spectrum of α-mycolate (Test), a similar phenomenon might have been expected for the meromycolate, but the relevant portions of the mass spectrum of methyl α-meromycolate (Test) were not produced.

Since methyl mycolate-I and methyl α-mycolate (Test) appear to be virtually identical in all respects,[1,3] the components of methyl α-mycolate (Test) giving rise to the meroaldehydes of *m/e* 740 and 768 probably have the structures (I, $x = 19$, $y = 15$, $z = 10$) and (I, $x = 19$, $y = 15$, $z = 12$), including the stereochemical assignments, the latter structure representing the main constituent of this ester. Recently, Etémadi[10] has mentioned a revised structure with $y = 14$ and $z = 13$ for α-mycolate (Test) based on a biogenetic analogy with the mycolic acids of *M. smegmatis* but evidence for this is not yet available.

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